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Developing spring type *Brassica napus* lines containing single clubroot resistance genes

Fengqun Yu
Plant Pathology, Saskaton Research and Development Centre, AAFC

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Canada

Major clubroot differential systems - pathotypes

1. Williams,1966: 2 cabbages (*B. oleracea*) and 2 rutabaga including ECD10 (*B. napus*)

2. Buczacki, et al 1975: 5 lines of *B. rapa* (ECD01 to ECD05), 5 lines of *B. napus* (ECD06-ECD10) and 5 lines of *B. oleracea* (ECD11-ECD15)

3. Some et al, 1996: 7 lines of *B. napus* including ECD06, ECD07-ECD10 and 2 lines of *B. oleracea* (ECD11 and ECD13)

4. Strelkov et al, 2018: 2 lines of *B. rapa* (ECD02, ECD05), 9 lines of *B. napus* including ECD06, ECD07-ECD10 and 2 lines of *B. oleracea* (ECD11 and ECD13)

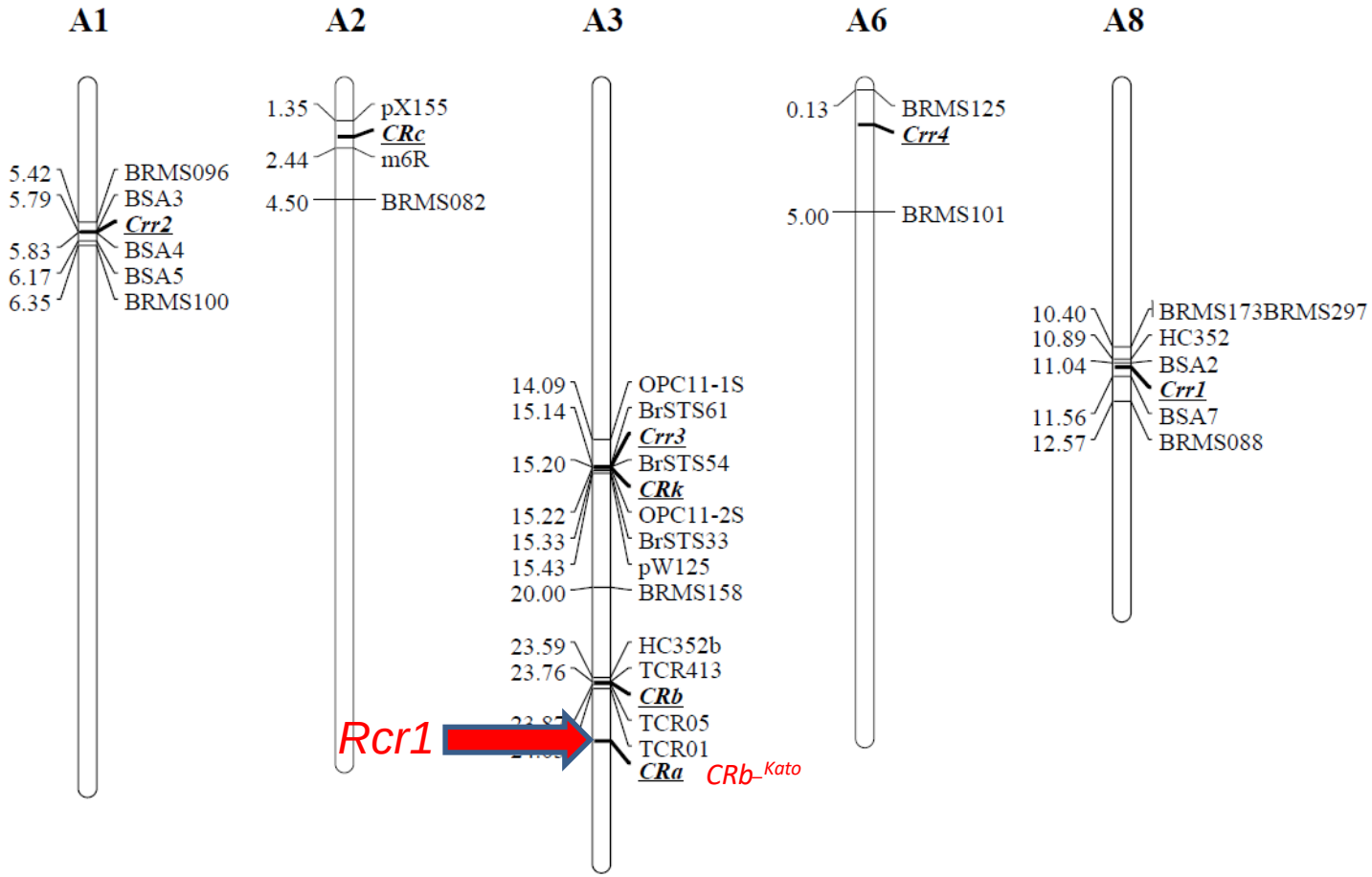
Drawbacks on the differential systems for canola in Canada

- Most of the lines are vegetable or fodder Brassica crops.
- Vernalization is needed.
- They cannot grow well in canola fields in western Canada.
- Inheritance for resistance to clubroot in most of the resistant lines is not clear.

Spring type *Brassica napus* near isogenic lines

- A set of near-isogenic spring canola lines containing single clubroot resistance genes would be the best differential lines.
- We proposed developing near isogenic in 2013 and the project was initiated in April 1, 2014.
- Applications in clubroot management
  - Differentiate races of *P. brassicae*
  - Monitor changes in race structure of the pathogen population in canola fields
  - Canola germplasm for resistance to clubroot
- What did we need?
  - Identified and mapped clubroot resistance (CR) genes
  - Isolates or strains that can differentiate each CR gene
  - Molecular markers for selection

Identified and mapped CR genes in *B. rapa* by 2013



Piao, 2014

Collecting materials for the project

Donor-R gene	Type	Provider	Isolates
Siloga -*Crr1, Crr2* and *Crr4*	Turnip	Centre for Genetic Resources, the Netherlands	Not available
Milan White-*Crr3*	Turnip	Italian Seed and Tool, USA	Not available
ECD02-*CRa*	Turnip	University of Warwick, UK	Not available
ECD01-*CRb*	Turnip	University of Warwick, UK	Not available
Debra-*CRc* and *CRk*	Turnip	Centre for Genetic Resources, the Netherlands	Not available
Flower Nabana- *Rcr1*	Pak choy	Evergreen Y.H. Enterprises, USA	Dr. Strelkov

Except *Rcr1*, no SNP markers were available when the project was initiated.

Challenges on developing near isogenic lines

Developing canola isogenic lines for clubroot is much more difficult than developing the lines for blackleg.

- Interspecific crosses
- Vernalization needed (4-6 months)
- No differential isolates
- No molecular markers available for most of the CR genes

Breeding method

B. napus DH16516 $A^dA^dC^dC^d$ **X** *B. rapa* vegetables A^vA^v

DH16516 **X** Triploid $A^dA^vC^d$

DH16516 **X** BC1 A^d $A^d/A^vC^d[C^{d\ (0\text{-}9)}]$

DH16516 **X** BC2

BC3

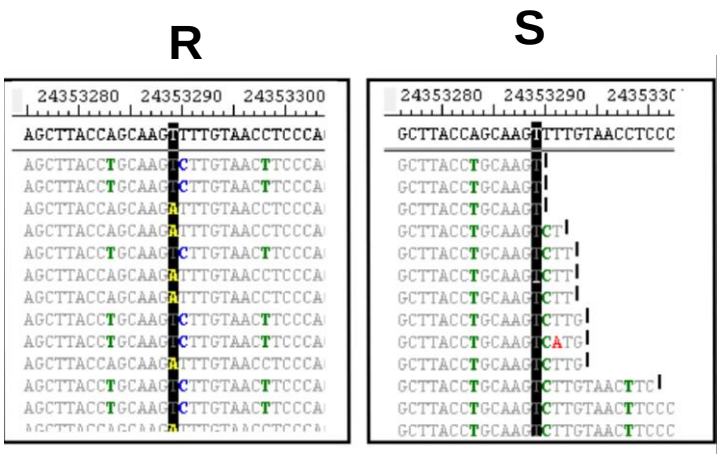
- Transfer resistance genes from *B. rapa* vegetables into *B. napus*
- Recover full set of C-genome chromosomes (9 pairs)
- Eliminate unnecessary genetic background from the vegetables

Backcrosses until BC4 and self-pollination to produce BC4S2 (*Rcr1*)

Microspore culture for developing doubled haploids (the rest of R genes)

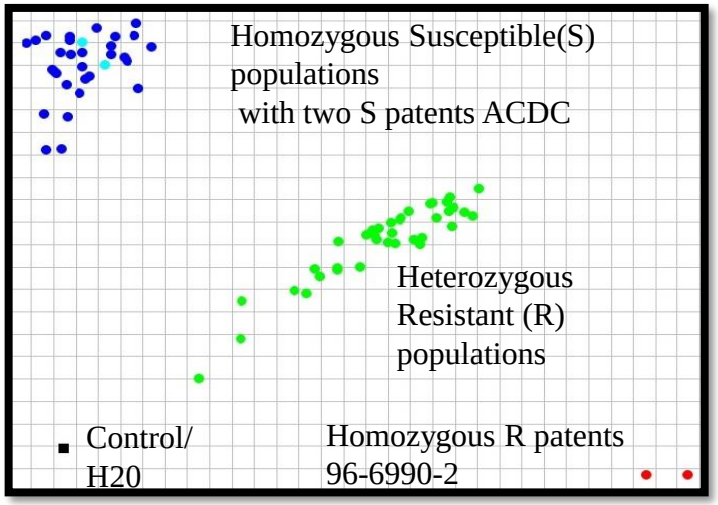
Identifying and genotyping SNP

R and S bulks



Discovery of polymorphic variants (SNP and InDel)

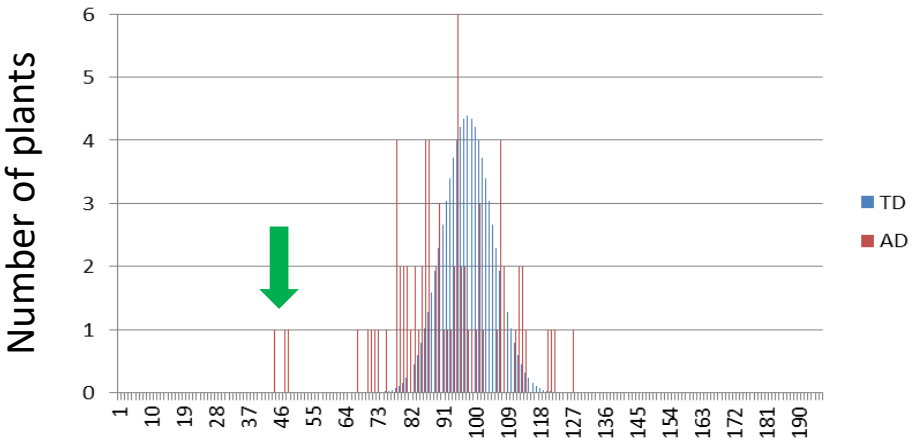
- 23 SNP markers were tightly associated with resistance to pathotype 3H with *Rcr3* on A03 in 240 BC_1 plants



Kompetitive Allele-Specific PCR (KASP)

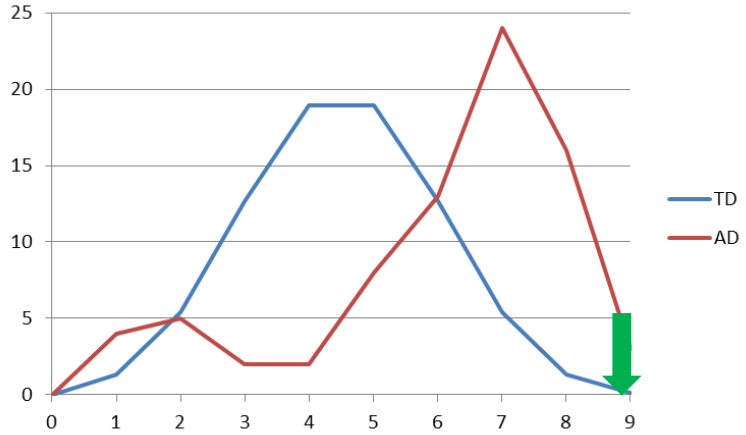
Introgression of *Rcr1* gene into canola

Analysis of A-genome markers genome-wide SNP marker analysis using 6K Illumina SNP assay



Number of markers from the CR donor FN

Analysis of C-genome markers



Number of C genome chromsomes

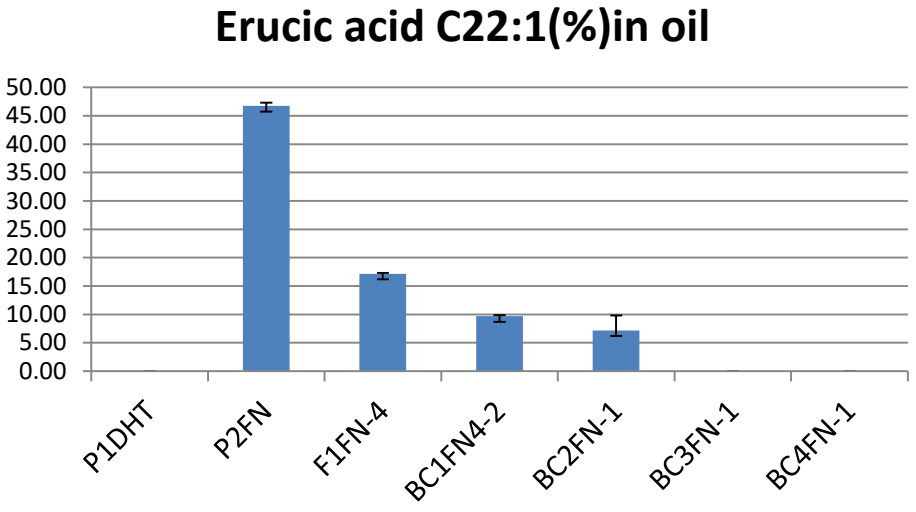
- A total of 77 CR BC1 plants
- 198 polymorphic robust markers almost evenly distributed on A-genome chromosomes
- Theoretical distribution (TD): binomial; parameters *N* = 198 and *P* = 50%
- Actual distributions (AD)

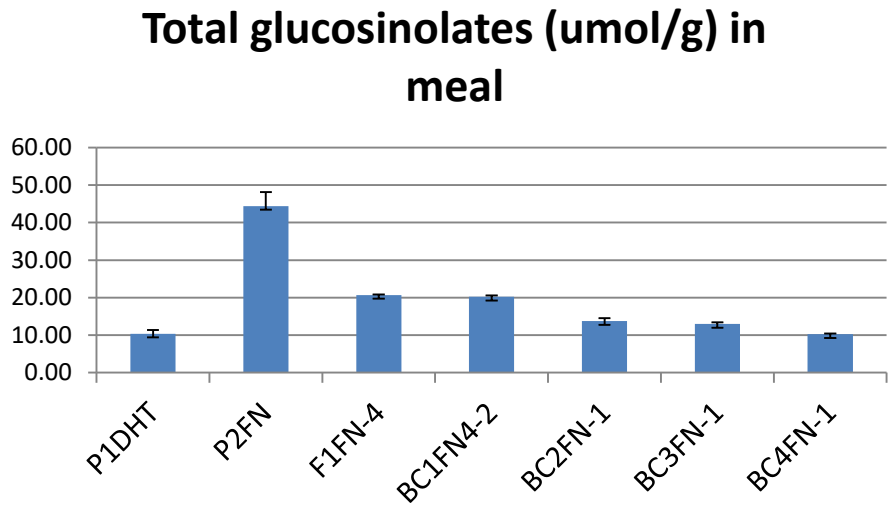
- Theoretical distribution (TD): binomial; parameters *N* = 9 and *P* = 50%; average = 4.5
- Actual distributions (AD): More plants with 6 to 9 C-genome chromosomes; average = 6.1
- High frequency of C-genome transmission

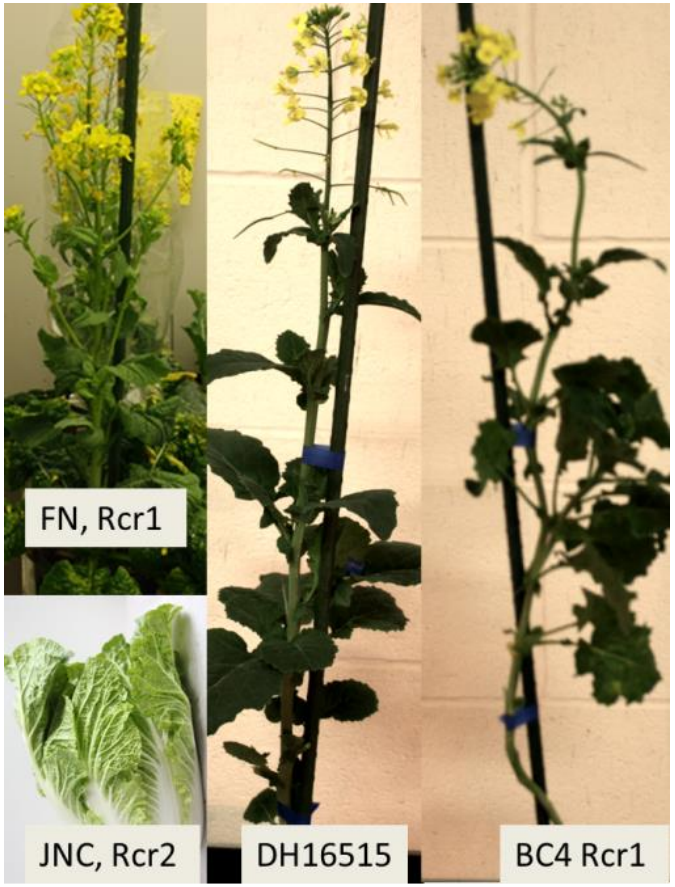
Evaluation of resistance to pathotype 3 in the *B. napus* lines of DH16516 x *B.rapa* FN (*Rcr1*)

Lines	Generation	Total	R	S
DH16516	*B. napus* recipient	17	0	17
FN	*Rcr1 B.rapa* donor	7	7	0
BC2FN		16	10	6
BC2S2FN-1	BC2S2	16	16	0
BC2S2FN-2	BC2S2	12	12	0
BC2S2FN-3	BC2S2	12	12	0
BC2S2FN-4	BC2S2	13	13	0
BC3FN-3	BC3	24	9	12
BC4FN-1	BC4	34	17	17
BC4S1FN	BC4S1	32	23	9

Canola quality plants carrying *Rcr1* and morphologically similar to DH16516

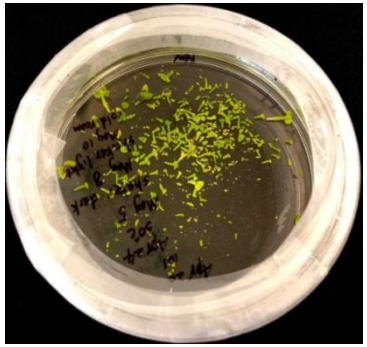






Progress on developing spring type *B. napus* carrying single CR genes

Donor Line	CR gene	Chr	Generation	No. of DH plants developed
Turnip Milan White	*Crr3*	A03	BC3	embryos
ECD01	*CRb*	A03	BC3	50
ECD02	*CRa*	A03	BC3	100
Debra	*CRc* *CRk*	A02 A03	BC2/BC3	100 lines
Siloga	*Crr1* *Crr2* *Crr4*	A08 A01 A06	BC3	200



Selecting spring type DH plants containing single R genes

- Increase seed each DH line
- Test for resistance to different strains
- Observe traits including days to flower
- Confirm the presence of each CR gene with SNP markers
- Identify additional genes

Selecting spring type DH plants containing single R genes from ‘Debra’ (59 out of 100 DH lines)

R to pathotypes	3	5X
R	32	28
S	27	31
P (1:1)	0.515	0.696

Single genes control resistance to pathotypes 3 and 5X respectively

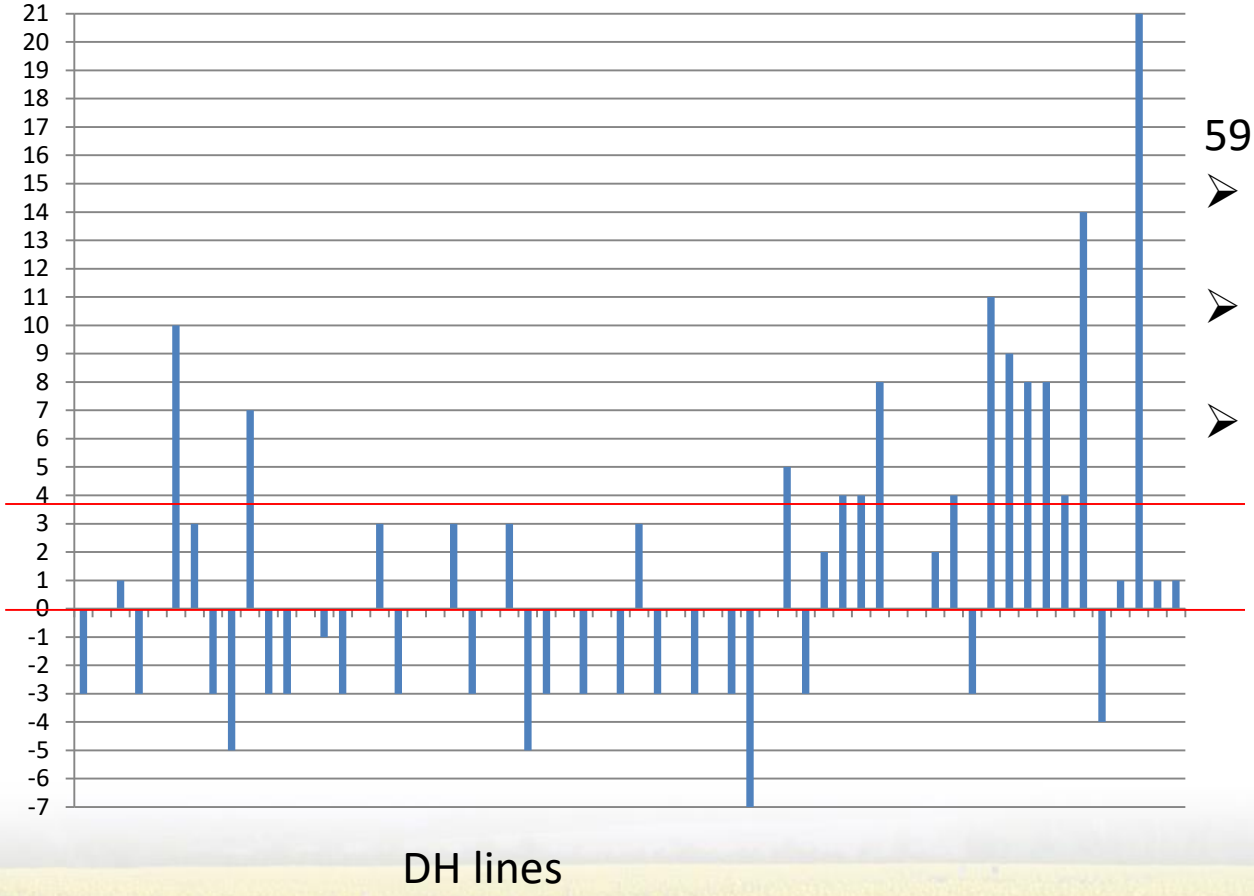
Which genes are there in the DH lines from 'Debra' ?

Debra	*CRc*	A02
	CRk?	A03

Phenotype (3, 5X)	**No. of lines**
R, R	23
R, S	9
S, R	5
S, S	22
P (1:1:1:1)	0.0007536

- CR genes for resistance to pathotypes 3 and 5X are linked.
- Resistance to pathotype 3 may be controlled by *CRc* (A02).
- Resistance to pathotype 5x may be controlled by a second gene on chromosome A02 (*Rcr8?*).
- Confirming presence of the CR genes is in progress.

Days to flower comparing with the recipient *B. napus* line DH16516



59 lines:

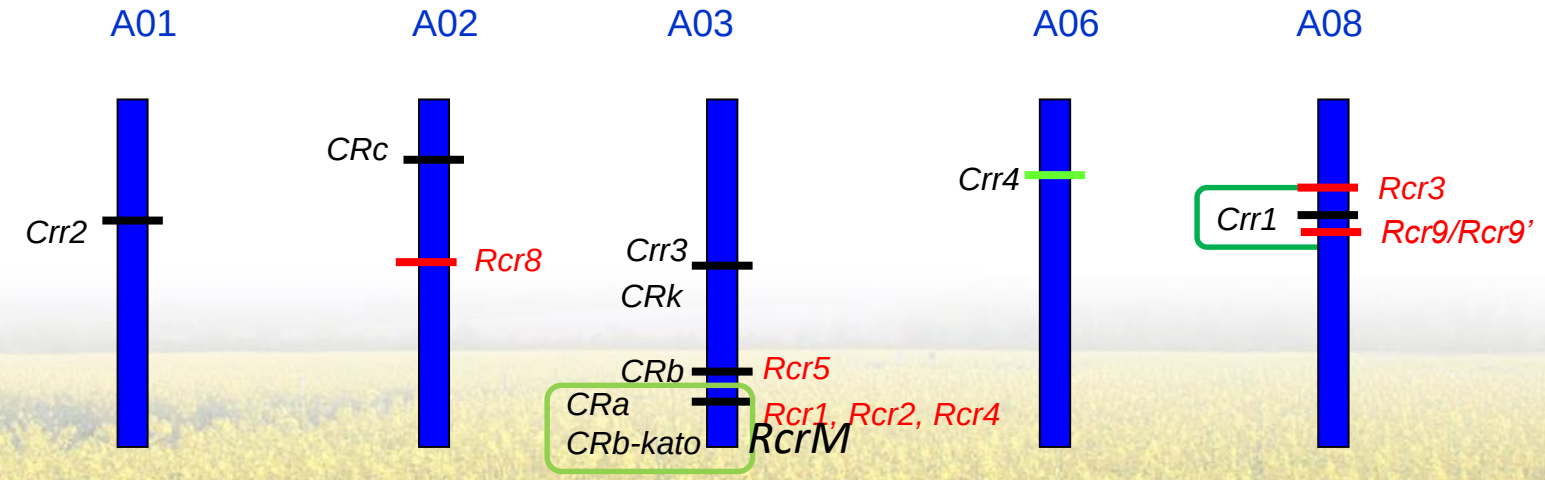
- No vernalization is needed for all of the lines.
- 14 lines was the same as DH16516
- >3 days delay in 9 lines

Selecting DH lines containing single CR genes

Line	3	5X	R gene	Days to flower (DF)	DF more or less than DH16516
YDH98-026	R	S	*CRc?*	42	-3
YDH98-033	R	S		44	-1
YDH98-041	R	S		45	0
YDH98-009	R	S		45	0
YDH98-084	R	S		42	-3
YDH98-087	R	S		45	0
YDH98-051	R	S		45	0
YDH98-074	R	S		58	4
YDH98-100	R	S		50	-4
YDH98-019	S	R	*Rcr8?*	55	10
YDH98-016	S	R		45	0
YDH98-055	S	R		48	3
YDH98-058	S	R		45	0
YDH98-047	S	R		54	0
DHT	S	S		45	
DHT	S	S		54	
Debra	R	R			
Debra	R	R			

How many CR genes are there in *B. rapa*?

CR gene/QTL	Species	Type	Cultivar	Chr	R to pathotype	Markers
Rcr1	*B. rapa*	Pak choy	Flower Nabana	A03	2, 3, 5, 8	SSR, SNP
Rcr2	*B. rapa*	Chinese cabbage	Jazz	A03	2, 3, 5, 8	SNP
Rcr3	*B. rapa*	Oilseed rape	96-6990	A08	3	SNP
Rcr4	*B. rapa*	Oilseed rape	T19	A03	2, 3, 5, 6, 8	SNP
Rcr5	*B. rapa*	Turnip	PTWG	A03	3	SNP
Rcr6 /Rcr6'	*B. nigra*	Black mustard	PI /CR2716	B3	3, 5X	SNP
Rcr7	*B.oleracea*	Cabbage	Tekila	C7	3, 5X	SNP
Rcr8	*B. rapa*	Oilseed rape	T19	A02	5X	SNP
Rcr9	*B. rapa*	Oilseed rape	T19	A08	5X	SNP
Rcr9'	*B. rapa*	Oilseed rape	96-6990	A08	5X	SNP



Identified CR genes in diploid species of *Brassica*

R gene	Chr	Single R gene in DH16516
Crr2	A01	under development
CRc	A02	yes
Rcr8	A02	yes?
CRa/CRb$_{Kato}$*/Rcr1/Rcr2/Rcr4/RcrM* ?	A03	yes
Crr3/CRk ?	A03	under development
CRb/Rcr5 ?	A03	under development
CRd	A03	Not started
Crr4	A06	?
Rcr3	A08	under development
Crr1	A08	under development
RCr9/Rcr9' ?	A08	under development
Rcr6 /Rcr6' ?	B3	under development
Rcr7	C7	under development

Spring type *B. napus* lines containing each of 8 genes becomes available in two years (2^8=256).

- Very informative differential set: 2^{10}=1024; 2^{11}=2048; 2^{12}=4096; 2^{13}=4192

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